needs to achieve to exploit the anti-inflammatory effect of FICZ, observed in certain experimental models? Many individuals with precancerous lesions continue to expose themselves to the sun. It remains to be seen how this photosensitizer contributes to skin carcinogenesis in such cases. Furthermore, it is still uncertain how much UV exposure is required for its beneficial or detrimental effects in a human setting. As the bulk of the studies performed so far have used single wavelength, future studies employing a combination of UVA and UVB simulating the solar UV spectrum will provide better insight into the mechanism(s) involved.

In summary, this study contributes to our understanding of the role of endogenous photosensitizers in the human skin. Rigorous characterization of the novel chromophore FICZ in response to different wavelengths of UV spectrum and the molecular mechanisms involved in photooxidative stress will reveal innovative opportunities for its exploitation in cutaneous pathologies.

CONFLICT OF INTEREST

The authors state no conflict of interest.

REFERENCES

Afaq F, Zaid MA, Pelle E *et al.* (2009) Aryl hydrocarbon receptor is an ozone sensor in human skin. *J Investig Dermatol Symp Proc* 129:2396–403

Anderson LM, Jones AB, Riggs CW *et al.* (1989) Modification of transplacental tumorigenesis by 3-methylcholanthrene in mice by genotype at the Ah locus and pretreatment with beta-naphthoflavone. *Cancer Res* 49:1676–81

Archier E, Devaux S, Castela E *et al.* (2012) Carcinogenic risks of psoralen UV-A therapy and narrowband UV-B therapy in chronic plaque psoriasis: a systematic literature review. *J Eur Acad Dermatol Venereol* 26(Suppl 3): 22–31

Bergander L, Wincent E, Rannug A *et al.* (2004) Metabolic fate of the Ah receptor ligand 6-formylindolo[3,2-b]carbazole. *Chem Biol Interact* 149:151–64

Castano AP, Demidova TN, Hamblin MR (2004) Mechanisms in photodynamic therapy: part one-photosensitizers, photochemistry and cellular localization. *Photodiagnosis Photodyn Ther* 1:279–93

Di Meglio P, Duarte JH, Ahlfors H *et al.* (2014) Activation of the aryl hydrocarbon receptor dampens the severity of inflammatory skin conditions. *Immunity* 40:989–1001

Fritsche E, Schafer C, Calles C *et al.* (2007) Lightening up the UV response by identification of the arylhydrocarbon receptor as a cytoplasmatic target for ultraviolet B radiation. *Proc Natl Acad Sci USA* 104:8851–6

Gniadecki R, Christoffersen N, Wulf HC (2002) Cholesterol-rich plasma membrane domains (lipid rafts) in keratinocytes: importance in the baseline and UVA-induced generation of reactive oxygen species. *J Investig Dermatol Symp Proc* 118:582–8

Hiraku Y, Ito K, Hirakawa K *et al.* (2007) Photosensitized DNA damage and its protection via a novel mechanism. *Photochem Photobiol* 83: 205–12

Klecak G, Urbach F, Urwyler H (1997) Fluoroquinolone antibacterials enhance UVA-induced skin tumors. *J Photochem Photobiol* 37:174–81

Ma Q (2011) Influence of light on aryl hydrocarbon receptor signaling and consequences in drug metabolism, physiology and disease. *Expert Opinion Drug Metab Toxicol* 7:1267–93

Magiatis P, Pappas P, Gaitanis G *et al.* (2013) Malassezia yeasts produce a collection of exceptionally potent activators of the Ah (dioxin) receptor detected in diseased human skin. *J Investig Dermatol Symp Proc* 133:2023–30

Mahmoud BH, Hexsel CL, Hamzavi IH *et al.* (2008) Effects of visible light on the skin. *Photochem Photobiol* 84:450–62

Nair S, Kekatpure VD, Judson BL *et al.* (2009) UVR exposure sensitizes keratinocytes to DNA adduct formation. *Cancer Prev Res* 2:895–902

Nayak CS (2005) Photodynamic therapy in dermatology. *Indian J Dermatol Venereol Leprol* 71: 155–60

Oberg M, Bergander L, Hakansson H *et al.* (2005) Identification of the tryptophan photoproduct 6-formylindolo[3,2-b]carbazole, in cell culture medium, as a factor that controls the background aryl hydrocarbon receptor activity. *Toxicol Sci* 85:935–43

Park SL, Justiniano R, Williams JD *et al.* (2015) The tryptophan-derived endogenous aryl-hydrocarbon receptor ligand 6-formylindolo[3,2-B]carbazole (Ficz) is a nanomolar uva-photosensitizer in epidermal keratinocytes. *J Invest Dermatol* 135: 1649–58

Pouget JP, Douki T, Richard MJ *et al.* (2000) DNA damage induced in cells by gamma and UVA radiation as measured by HPLC/GC-MS and HPLC-EC and Comet assay. *Chem Res Toxicol* 13:541–9

Uno S, Dalton TP, Shertzer HG *et al.* (2001) Benzo[a]pyrene-induced toxicity: paradoxical protection in Cyp1a1(-/-) knockout mice having increased hepatic BaP-DNA adduct levels. *Biochem Biophys Res Commun* 289: 1049–56

Wei YD, Rannug U, Rannug A (1999) UV-induced CYP1A1 gene expression in human cells is mediated by tryptophan. *Chem Biol Interact* 118:127–40

Wincent E, Bengtsson J, Mohammadi Bardbori A *et al.* (2012) Inhibition of cytochrome P4501-dependent clearance of the endogenous agonist FICZ as a mechanism for activation of the aryl hydrocarbon receptor. *Proc Natl Acad Sci USA* 109:4479–84

Wondrak GT, Jacobson MK, Jacobson EL (2006) Endogenous UVA-photosensitizers: mediators of skin photodamage and novel targets for skin photoprotection. *Photochem Photobiol Sci* 5: 215–37

See related article on pg 1598

Hairy Math: Addition of Wnt-3a to Multiply Bulge Cells

M. Zulfiquer Hossain[1] and Luis A. Garza[1]

Canonical Wnt signals are important for activation of epithelial skin stem cells, but the role of individual Wnt ligands remains uncertain. Ouji *et al.* demonstrate a key role for Wnt-3a in partial maintenance and long-term expansion of epithelial skin stem cells *in vitro*. They also report a method for expanding these cells *in vitro* without feeder cells.

Journal of Investigative Dermatology (2015) **135,** 1481–1483. doi:10.1038/jid.2014.545

The role of canonical Wnt-β-catenin signaling in hair follicle and epithelial stem cell biology has intrigued investigators for years. Numerous studies have implicated this signaling cascade in different aspects of hair

[1]*Department of Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA*

Correspondence: Luis A. Garza, Department of Dermatology, Johns Hopkins University School of Medicine, CRB II Suite 204, 1551 Orleans Street, Baltimore, Maryland 21231, USA. E-mail: LAG@jhmi.edu

Clinical Implications

- Wnt-3a supports partial maintenance and long-term expansion of epithelial bulge stem cells in the absence of feeder cells *in vitro*.
- Distinct Wnt ligands might have different functions in the skin.
- Expansion of bulge cells with Wnt-3a may emerge as a strategy for cellular therapy of hair loss.

follicle morphogenesis, regulation of the hair follicle cycling, and promotion of hair growth (Lien and Fuchs, 2014). However, given the large number of mammalian Wnt ligands (19) and frizzled (Fzd) receptors (10) in the context of a complex interplay between activating and inhibiting molecules within this pathway (Clevers *et al.*, 2014), the precise role of individual Wnt molecules remains largely undefined. In the current issue of the *Journal of Investigative Dermatology*, Ouji *et al.* (2015) shed light on the role of Wnt-3a in partial maintenance and long-term expansion of bulge stem cells *in vitro*.

The canonical Wnt-β-catenin pathway is among the most highly conserved signaling cascades in mammalian development, organogenesis, and tissue homeostasis (Clevers *et al.*, 2014). In the absence of a canonical Wnt signal, a degradation complex, consisting of APC, axin, and GSK-3β, sequesters cytosolic β-catenin. In this complex, GSK-3-β kinase phosphorylates β-catenin at the N-terminus and marks it for ubiquitylation and proteasomal degradation. Simultaneously, members of the lymphocyte enhancement factor/T-cell factor (LEF/TCF) family of transcription factors keep Wnt target genes inactive by interacting with TLE (Groucho) corepressor protein on promoters. The binding of a member of the Wnt family of secreted glycoproteins to the Fzd receptors and the LRP coreceptors on the cell surface leads to inactivation of GSK-3-β. As a result, β-Catenin is no longer phsosphorylated or degraded. Nonphosphorylated β-catenin accumulates in the cytoplasm, translocates to the nucleus, binds to LEF/TCF, directly displaces TLE, and induces expression of Wnt-β-catenin target genes. This signaling pathway can be modulated by a number of extracellular inhibitory molecules. These include the DKK family of secreted proteins, which interact with LRP6 co-receptor and Kremen, secreted frizzled-related proteins, which, in turn, compete with Fzd receptors for Wnt binding, and with Wif-1, which also binds Wnt molecules. Canonical Wnt-β-catenin signaling has a critical role in regulating proliferation, survival, and differentiation of numerous cell types. For example, β-Catenin induces telomerase expression directly and, thus, may promote cell lineage longevity (Clevers *et al.*, 2014).

Hair follicles regenerate throughout an animal's lifetime through periodic activation of long-lived stem cells in the bulge region. The hair follicle cycle consists of a growth phase (anagen) when the bulge stem cells divide to self-perpetuate and to give birth to progenitor cells that regenerate the hair follicle, an involuting phase (catagen) when the lower two-thirds of the hair follicle shrinks, and a resting phase (telogen; Choi *et al.*, 2013). When the bulge is activated to divide during anagen, most of the bulge stem cells (about 60%) do not contribute to the next episode of hair growth but, rather, remain in the bulge. However, a significant fraction of these cells (about 25%) are lost, and the rest leave the bulge to produce lineages primarily in the relatively undifferentiated outer root sheath (Rompolas *et al.*, 2013). Clearly, even under the best circumstances, bulge stem cells have a somewhat limited capacity for expansion, because they must contribute to the hair follicle, and when they divide a fairly large portion are typically lost. Therefore, long-term, high multiplicity, *in vitro* expansion of bulge stem cells presents a formidable technical challenge. Recent reports have shown that canonical Wnt-β-catenin signals are necessary for activation and proliferation of hair follicle stem cells, but these signals are not necessary for their maintenance (Choi *et al.*, 2013; Deschene *et al.*, 2014; Lien *et al.*, 2014). In a separate study, Wnt-7b was reported to be required for activation of bulge stem cells and hair follicle cycling (Kandyba and Kobielak, 2014). However, more work is required to characterize the roles of specific Wnts in hair biology.

In the accompanying article, Ouji *et al.* (2015) set out to investigate the role of Wnts in the generation and maintenance of bulge stem cells. More specifically, they examined whether distinct Wnt ligands have different functions and whether individual Wnt molecules act through distinct or redundant mechanisms. They also tested whether it is possible not only to maintain but also to multiply bulge stem cells *in vitro*, both in the long term and without 3T3 fibroblast feeder cells. CD34 has been established as a unique marker for mouse hair follicle stem cells in the bulge region. Also, although CD49f (α6 integrin) is not expressed exclusively in bulge stem cells, bulge stem cells are known to express a very high level of this cell surface molecule as well (Trempus *et al.*, 2003). The authors of the current study, therefore, first verified that freshly isolated CD49f + CD34+ cells were indeed bona fide epithelial stem cells (EpSC). CD49f+ CD34+ cells expressed other stem cell markers such as Lhx2, Lgr, Keratin 15, Sox9, S100a6, and NFATc1. However, they did not express differentiated epithelial cell markers: Keratin 1, Keratin 2, Loricrin, mHa5, or mHb5. Furthermore, these cells recapitulated hair follicle development and hair growth after cotransplantation with dermal fibroblasts in nude mice. Next, the investigators showed that Wnt-3a, but not Wnt-5a, Wnt-10b, or Wnt-11, mediated partial maintenance and long-term expansion of CD49f+ CD34+ EpSCs *in vitro*, without any requirement for feeder cells. At the end of 10 days in culture with Wnt-3a, about 8–10% of the cell population remained CD49f+ CD34+. These cells were sorted and subjected to a second 10-day culture, and the entire procedure was repeated 15 times. Strikingly, CD49f+ CD34+

cells sorted from each sequential culture retained the same stem cell characteristics as the original cells. Importantly, CD34+ cells express Wnt-3a, whereas CD34- cells express Wnt-inhibitors Dkk-1 and Wif-1, indicating a possible role of autocrine Wnt-3a signaling in maintenance and expansion of EpSCs. Wnt-3a also delayed conversion of CD34+ cells to the CD34- phenotype and reduced the expression of Dkk-1 and Wif-1. Consistent with this, suppression of Dkk-1 activity with a neutralizing antibody against Dkk-1 partially restored Wnt-responsiveness of EpSc cultures. Collectively, these data showed that Wnt-3a has the potential to support partial maintenance and long-term expansion of bulge stem cells *in vitro*. Intriguingly, in contrast to Wnt-3a, Wnt-10b promoted keratinocyte differentiation as evident from the induction of differentiation markers and suppression of stem cell markers. Despite this difference in biologic effects, Wnt-3a and Wnt-10b both induced β-catenin-TCF-dependent gene expression in the TOPFLASH reporter assay, yet activated entirely different sets of genes in keratinocytes.

The findings from the present study advance our understanding of *in vitro* maintenance and expansion of bulge stem cells and the role of individual Wnt ligands in this process in several important ways. First of all, the authors succeeded in developing a method for culturing bulge stem cells *in vitro* without feeder cells. Their study suggests an important role for Wnt-3a in the maintenance of bulge stem cells. Finally, it shows that two different canonical Wnts (Wnt-3a and Wnt-10b in this study) can have entirely different gene expression profiles in the same cells, even when they both activate β-catenin-TCF-dependent gene expression. Given the comprehensive nature of this investigation, it is unfortunate that Wnt-7b, which has been implicated in the activation of bulge stem cells (Kandyba and Kobielak, 2014), was not studied. It is also important to note that only a few extra cells (about 5–10%) are actually maintained as bulge stem cells as a result of Wnt-3a treatment over controls. This very modest increase in CD34+ cell number becomes critical only after the culture without Wnt-3a loses the entire bulge stem cell population by day 10. Because Wnt-3a-mediated maintenance of bulge stem cells is at best partial, it is imperative to investigate the role of other potential self-renewal factors.

This study raises other interesting questions. It is not entirely clear how Wnt-3a contributes to maintenance of the bulge stem cell population. For example, what Wnts can compensate *in vivo* in Wnt-3a hypomorphic mice? It will be quite useful to examine how distinct Wnt ligands induce different gene expression profiles in the same cells even as they signal through the same transcriptional co-factor (β-catenin). Similarly, it will be exciting to determine how the same Wnt molecules induce different gene expression profiles in different cells. Epigenetic mechanisms might hold the key to these unresolved questions. Nevertheless, the current study is a timely and insightful demonstration of the important role Wnt signals have in the generation and expansion of stem cell populations.

CONFLICT OF INTEREST

The authors state no conflict of interest.

REFERENCES

Choi YS, Zhang Y, Xu M *et al.* (2013) Distinct functions for Wnt/beta-catenin in hair follicle stem cell proliferation and survival and interfollicular epidermal homeostasis. *Cell Stem Cell* 13:720–33

Clevers H, Loh KM, Nusse R (2014) Stem cell signaling. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. *Science* 346:1248012

Deschene ER, Myung P, Rompolas P *et al.* (2014) beta-Catenin activation regulates tissue growth non-cell autonomously in the hair stem cell niche. *Science* 343:1353–6

Kandyba E, Kobielak K (2014) Wnt7b is an important intrinsic regulator of hair follicle stem cell homeostasis and hair follicle cycling. *Stem Cells* 32:886–901

Lien WH, Fuchs E (2014) Wnt some lose some: transcriptional governance of stem cells by Wnt/beta-catenin signaling. *Genes Dev* 28: 1517–32

Lien WH, Polak L, Lin M *et al.* (2014) In vivo transcriptional governance of hair follicle stem cells by canonical Wnt regulators. *Nat Cell Biol* 16:179–90

Ouji Y, Ishizaka S, Nakamura-Uchiyama F *et al.* (2015) Partial maintenance and long-term expansion of murine skin epithelial stem cells by Wnt-3a in vitro. *J Invest Dermatol* 135: 1598–608

Rompolas P, Mesa KR, Greco V (2013) Spatial organization within a niche as a determinant of stem-cell fate. *Nature* 502:513–8

Trempus CS, Morris RJ, Bortner CD *et al.* (2003) Enrichment for living murine keratinocytes from the hair follicle bulge with the cell surface marker CD34. *J Invest Dermatol* 120: 501–11